produced a dose-related reduction in mean arterial blood pressure when administered orally (Figure 1). At single doses of 0.1 and 0.3 mg/kg po, L-158,809 reduced the mean arterial blood pressure to normotensive levels (~60 and ~80 mmHg), with a duration of action exceeding 24 h. The onset of action occurred within 30 min, and the maximal response was measured at approximately 3 h. DuP 753 (1.0 mg/kg po) was more than 10-fold less potent and produced a maximal decrease in blood pressure at approximately 6 h (Figure 1). At the end of the protocol, iv bolus injections of 1.0 or 0.6 mg/kg enalaprilat were used to confirm the renin dependence of blood pressure in these animals, and the effect of these large doses was similar to response levels produced by L-158,809 at 0.3 mg/kg po. A hypotensive effect of L-158,809 or DuP 753 was not observed in normotensive rats with normal renin levels.

In summary, imidazo[4,5-*b*]pyridine analogues **1** represent an important new class of AII antagonists. The biological profile of L-158,809 (**1f**) indicates it to be a highly potent and specific antagonist of AII both in vitro and in vivo. The excellent selectivity, potency, duration of action, and oral absorption of L-158,809 suggests it to be a useful tool in assessing the therapeutic value of AII receptor antagonism as well as the diverse roles of the RAS.

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Registry No. **1a**, 135145-91-4; **1b**, 135145-92-5; **1c**, 135145-93-6; **1d**, 135145-94-7; **1e**, 135145-95-8; **1f**, 135145-96-9; **2**, 106-38-7; **3**, 114772-53-1; **4**, 133051-88-4; **5a**, 4214-75-9; **5b**, 21901-29-1; **5c**, 6635-86-5; **5d**, 22934-23-2; **6a**, 68175-09-7; **6b**, 135145-97-0; **6c**, 133239-98-2; **6d**, 133052-13-8; **6e**, 135070-89-2; **6f**, 133240-06-9; angiotensin II, 11128-99-7; 2-bromobenzonitrile, 2042-37-7; butyric acid, 107-92-6; valeric acid, 109-52-4; propionic acid, 79-09-4.

(13) The protocol for aortic coarction of the rats is described: Sweet, C. S.; Columbo, J. M.; Gaul, S. L. Central Antihypertensive Effects of Inhibitors of the Renin-Angiotensin System in Rats. *Am. J. Physiol.* 1976, *231*, 1794 and references cited therein.

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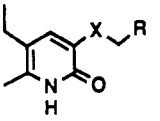
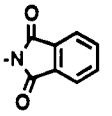
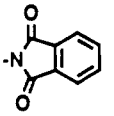
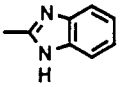
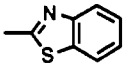
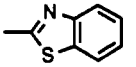
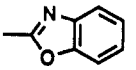
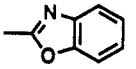
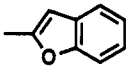
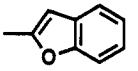
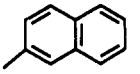
2-Pyridinone Derivatives: A New Class of Nonnucleoside, HIV-1-Specific Reverse Transcriptase Inhibitors

The development of potent and effective antiviral drugs for the control of human immunodeficiency virus type 1 (HIV-1) infection is one of the more pressing goals of contemporary medicinal chemistry. The unique nature of the replication cycle of retroviruses, such as HIV-1, offers a variety of potential areas for chemotherapeutic intervention.[1] One particularly important target is the viral reverse transcriptase (RT). Nucleoside analogues, including 3′-azidothymidine (AZT) and dideoxyinosine (ddI), which inhibit the process of reverse transcription are clinically useful drugs for the treatment of HIV-1 infection.[2] However, the utility of these nucleoside analogues is limited by the emergence of resistant viral strains[3] and by serious clinical side effects[4] which may be related

Table I. Inhibition of HIV-1 RT by 2-Pyridinones: Aromatic and Heterocyclic Derivatives



no.	X	R	IC_{50}, nM[a]
1	NH		30
2	CH_2		3700
3	NH		6400
4	NH		350
5	CH_2		370
6	NH		210
7	CH_2		22
8	NH		235
9	CH_2		77
10	NH		440

[a] The HIV-1 RT assay using rC·dG as template primer was carried out in a reaction mixture (50 μL) containing 55 mM Tris-HCl (pH 8.2), 30 mM KCl, 30 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mg/mL bovine serum albumin (BSA), 20 μg/mL $rC{\cdot}dG_{(12-18)}$ (Pharmacia), 50 μM EGTA, 8 μM [^{3}H]dGTP, 0.01% (v:v) Triton X-100, and 0.9 nM recombinant HIV-1 RT. The remainder of the procedure was performed as previously described.[9] The concentration that produced 50% inhibition (IC_{50}) is stated as the mean of at least three experiments.

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to inhibition of cellular DNA polymerases by these agents.

Attention is therefore now focused on the development of more specific, nonnucleoside HIV-1 RT inhibitors. The discovery of potent, nonnucleoside, HIV-1-specific RT inhibitors in three chemically diverse series has been described recently.[5-7] A separate report from our laboratories[5] discloses the biological and mechanistic characterization of the 2-pyridinone class of HIV-1 RT inhibitors. In this communication, we describe the chemistry and structure–activity relationships (SAR) in the 2-pyridinone series which led to the selection of candidates for clinical evaluation as HIV-1 replication inhibitors.

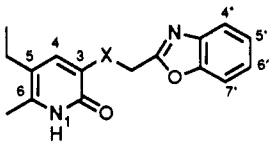
Phthalimide derivative 1 (Table I) was initially identified to be a potent and selective inhibitor of HIV-1 RT in an enzyme-based screening program. However, under in vitro physiological conditions, 1 is unstable and eliminates phthalimide with $t_{1/2} \approx 120$ min. Since (aminomethyl)-phthalimide derivatives such as 1 would appear to have limited clinical utility, different strategies were explored for the development of more useful analogues from this lead.

An initial attempt to improve stability involved replacement of the aminomethylene linker of 1 with ethylene to give 2. Although 2 proved to be hydrolytically stable, it was more than 100-fold weaker than 1 as an RT enzyme inhibitor. However it sufficiently inhibited the spread of HIV-1 infection in H9 human T-lymphoid cell culture experiments (CIC_{95} = 40 μM, Table III) to indicate that compounds of this structural class can effectively cross cell membranes and produce an antiviral effect.

An alternate approach which led to potent and stable inhibitors in both the aminomethylene and ethylene linker series involved replacement of the phthalimide moieties of 1 and 2 by various aromatic and heterocyclic groups (Table I). Of the compounds explored initially, the benzoxazole and benzofuran analogues (6 and 8, respectively) in the aminomethylene series exhibited high potency in the RT enzyme assay. Similar replacement of the phthalimide group of 2 with these heterocycles afforded the even more potent analogues 7 and 9 in the ethylene linker series. This result was unexpected since in the phthalimide series, aminomethylene analogue 1 is more active than the corresponding ethylene derivative 2.

The effect of introducing nuclear substituents into the benzoxazole moiety was investigated. Initial studies focused on the aminomethylene linker series. Systematic introduction of methyl groups into the benzoxazole ring of 6 identified the 4′- and 7′-positions as potency enhancing (Table II). In addition, disubstitution at these positions with either methyl or chloro (13 and 14, respectively) yielded even more potent RT inhibitors.

Table II. Inhibition of HIV-1 RT by Pyridinones: Benzoxazole Substituents



no.	X	substituent	IC_{50}, nM[a]
6	NH	none	210
11	NH	4′-Me	125
12	NH	7′-Me	55
13	NH	4′,7′-Me_2	20
14	NH	4′,7′-Cl_2	19
7	CH_2	none	22
15	CH_2	4′,7′-Me_2	23
16	CH_2	4′,7′-Cl_2	9.6

[a] See Table I, footnote *a* for assay procedure.

In the case of the ethylene linker analogue 7, methyl substitution in the 4′- and 7′-benzoxazole positions, 15, produced no change in enzyme inhibitory activity. Furthermore, introduction of chloro groups into these positions increased potency only by a factor of 2, 16 vs 7. These and other SAR results lead us to believe that although the aminomethylene and ethylene series of compounds may be occupying the same or overlapping sites on the enzyme-template-primer complex,[5] they are binding at that site in different orientations. Although 16 proved to be one of the more potent members of the series, it exhibited low oral bioavailability in animals and was not developed further.

Conversion of the pyridinone carbonyl of several of these compounds to thiocarbonyl led either to a modest 2-fold increase in potency or, more often, to a decrease in RT inhibitory activity. This is in contrast to the TIBO series of RT inhibitors where conversion of carbonyl to thiocarbonyl has been reported[6] to increase potency 450-fold.

Other changes in the pyridinone moiety did not lead to derivatives with improved activity. For example, alkylation of the pyridinone NH or elimination of the 6-methyl group (see Table II for numbering) reduced activity. The 5-ethyl group could be extended to *n*-propyl or *n*-butyl without much loss in potency, but shortening to methyl significantly reduced activity. Elimination of both 5- and 6-alkyl substituents abolished activity. Introduction of methyl into the 4-position of 6 yielded a 3-fold decrease in activity.

The aminomethylene and ethylene linker portions of these compounds were found to be very sensitive to changes in structure. For example, extension of the linker of 1 and 2 to three atoms or shortening it to one abolished activity. Also, conversion of the ethylene linker to either cis or trans olefin significantly reduced potency. RT inhibition was not improved by alkylation of the linker amino group.

Compounds that inhibited HIV-1 RT activity were evaluated for antiviral activity in cell culture. As shown in Table III, 7, 13, and 14 effectively inhibited the spread of HIV-1 infection in both MT-4 and H9 human T-lymphoid cell culture. No evidence of cytotoxicity was observed in these experiments at concentrations as high as 60 μM. Activities of BI-RG-587[7] and the TIBO analogue R82150[6] in our cell culture assay are also included in Table III for reference.

(4) Richman, D. D.; et al. The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. *New Engl. J. Med.* **1987**, *317*, 192–197. Dournon, E.; et al. Effects of Zidovudine in 365 Consecutive Patients with AIDS or AIDS-Related Complex. *Lancet* **1988**, II, 1297–1302.

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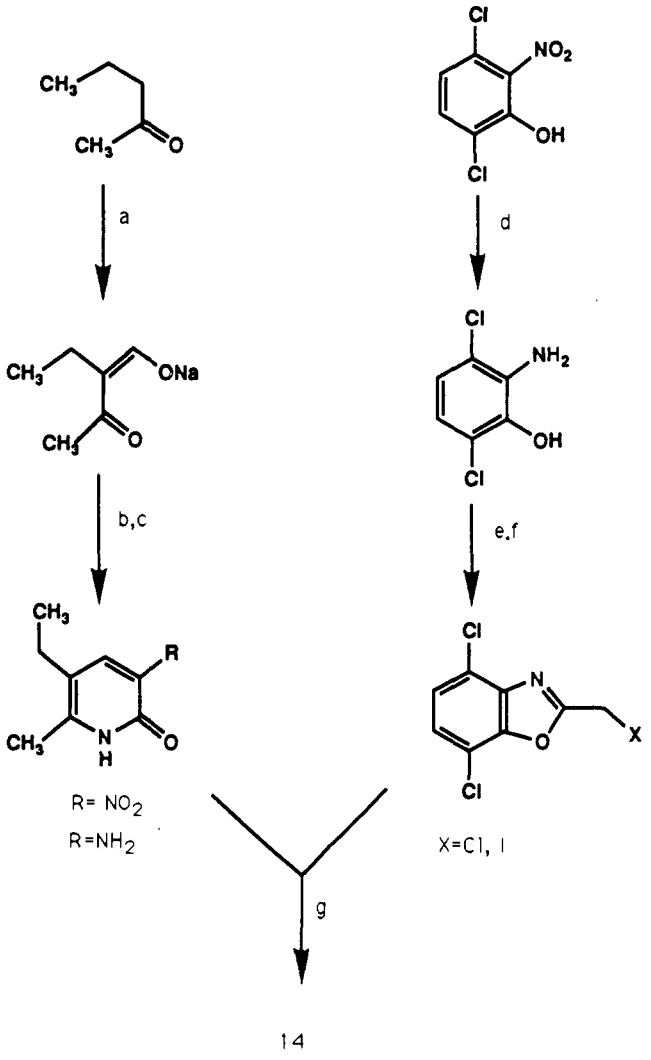
Scheme I[a]



[a] (a) HCO_2Et, NaOMe, $EtOH/Et_2O$, 44%; (b) $O_2NCH_2CONH_2$, aqueous piperidinium acetate, 80%; (c) H_2, Pd/C, 1:1 MeOH/THF, 68%; (d) H_2, Pt/C, EtOH/HOAc, quantitative; (e) ethyl chloroiminoacetate hydrochloride, CH_2Cl_2, 86%; (f) NaI, Me_2CO, 84%; (g) MeCN, $(i\text{-}Pr)_2NEt$, 42%.

The general synthetic routes to the 2-pyridinone ethylene and aminomethylene linker derivatives are illustrated by the preparations of 7 and 14 in Schemes I and II, respectively.[8]

The 2-pyridinones described in this communication constitute a novel series of potent, nonnucleoside, HIV-1-specific RT inhibitors structurally different from previously described inhibitors. Compounds 13 and 14 are currently undergoing phase I clinical studies to determine safety, tolerability, and pharmacokinetic parameters.

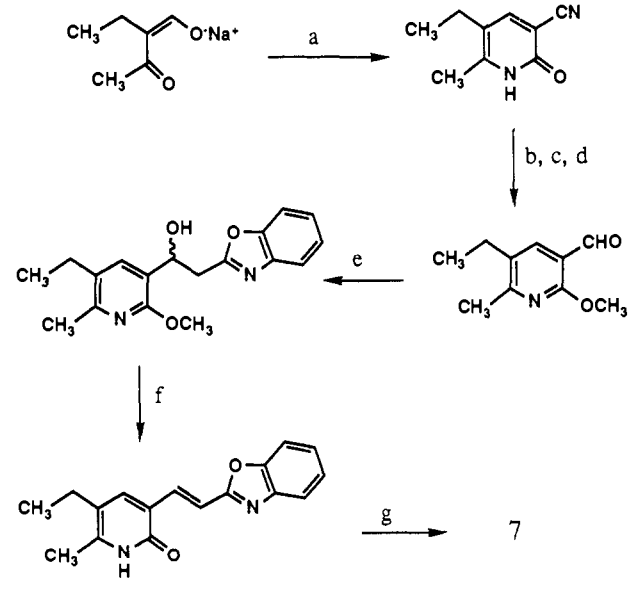
Acknowledgment. We thank W. Randall and J. Moreau for elemental analyses and J. Kaysen for manuscript preparation.

Table III. Antiviral Properties of Selected Compounds in Cell Culture

no.	CIC_{95}, nM[a] H9 cells[b]	CIC_{95}, nM[a] MT-4 cells[c]
2	40000	nd[f]
7	200	50–100
13	150	25–50
14	100	25–50
16	nd[f]	50–100
BI-RG-587[d]	nd[f]	100
R82150[e]	nd[f]	200

[a] Cell culture inhibitor concentrations (CIC_{95}) are defined as those which inhibited by > 95% the spread of HIV-1 infection in susceptible cell culture. [b] H9 cell assay: H9 human T-lymphoid cells[10] were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum. Antiviral assays were performed with use of HIV-1 strain IIIb.[11] Cells were grown in the presence of serial 2-fold dilutions of inhibitor in 96-well microtiter wells for one day prior to infection at low multiplicity (<0.001). Cultures were maintained in the continued presence of inhibitor for 13–14 days. At this time, culture supernatants were harvested and virus spread was assessed by HIV-1 p24 core antigen ELISA (Coulter Immunology). Indirect immunofluorescence assays using serum from an HIV-1-infected individual confirmed the direct relationship between p24 accumulation and the percentage of infected cells. Control cultures in the absence of inhibitor were fully infected within 7–10 days. [c] MT-4 cell assay: MT-4 human T-lymphoid cells[12] were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum. Cells were infected en masse at low multiplicity (0.01) with use of HIV-1 strain IIIb and were incubated for 24 h. At this time, cells were washed and distributed into 96-well microtiter dishes. Serial 2-fold dilutions of inhibitor were added to the wells and the cultures were maintained for 3 additional days. Virus spread was assessed by HIV-1 p24 core antigen ELISA. Control cultures in the absence of inhibitor were fully infected at 4 days. [d] Reference 7. [e] Reference 6. [f] Not determined.

Scheme II[a]



[a] (a) Cyanoacetamide, aqueous piperidinium acetate, 50%; (b) PCl_5, 120 °C, 61%; (c) CH_3ONa, CH_3OH, 82%; (d) Dibal-H, THF, 61%; (e) 2-methylbenzoxazole, *n*-BuLi, -100 °C, THF, 65%; (f) pyridine hydrochloride, 150 °C, 10 min, 80%; (g) H_2, Pd/C, CH_3OH/THF, 95%.

(8) All new compounds were characterized by satisfactory PMR spectra and elemental analysis.

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Supplementary Material Available: Experimental procedures, including analytical and spectial data, for the preparations of 7 and 14 (7 pages). Ordering information is given on any current masthead page.

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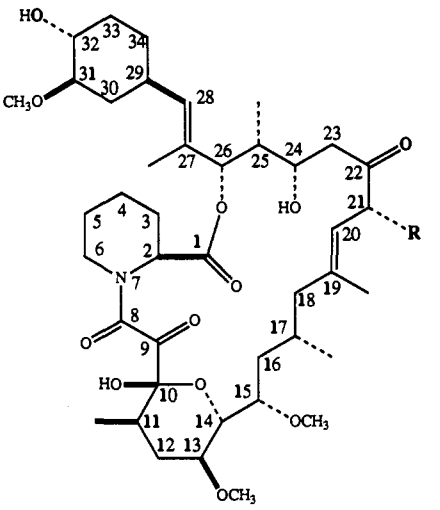
NMR Studies of an FK-506 Analogue, [U-^{13}C]Ascomycin, Bound to FKBP: Conformation and Regions of Ascomycin Involved in Binding

FK-506 is a potent immunosuppressant[1,2] that blocks T cell activation by inhibiting the production of lymphokines at the transcription level. Although its precise mechanism of action is unknown, its immunosuppressive activity appears to be related to the binding of FK-506 to a 108 amino acid protein from human T cells.[3,4] This FK-506 binding protein (FKBP) has been shown to be a peptidyl-prolyl cis-trans isomerase that is inhibited by FK-506[5,6] and the structurally related immunosuppressant, rapamycin.[6]

The X-ray crystal structure of FK-506 has been determined in the absence of FKBP[7] and used to aid in the design of FK-506 analogues.[8] In addition, the three-dimensional structure of FK-506 in chloroform solution has been recently obtained by NMR spectroscopy.[9] However,



FK-506 R=CH_2CHCH_2
Ascomycin R=CH_2CH_3

Figure 1. Structures of FK-506 and ascomycin.

as recently demonstrated by NMR studies of cyclosporin A bound to cyclophilin,[10-12] the conformation of a molecule when bound to its target site may be very different from its uncomplexed conformation determined in solution or in the crystalline state. In order to aid in the design of FK-506 analogues that are clinically useful as immunosuppressants, it would be of value to determine the conformation of FK-506 when bound to FKBP and to identify those portions of FK-506 that interact with the protein. To date, the ^{13}C carbonyl chemical shifts of C8 and C9[13] and the ^{1}H chemical shifts of the piperidine ring of FK-506 when bound to FKBP have been reported.[14] From the upfield shifts of the piperidine ring protons of FK-506 and rapamycin and NOEs observed between these protons and aromatic protons of FKBP, tentatively assigned to Trp59, Tyr89, and Phe99, it was concluded that the common pipecolinyl moiety of FK-506 and rapamycin is involved in binding to FKBP.[14]

In this report we describe NMR studies of a uniformly ^{13}C-labeled FK-506 analogue, ascomycin,[15] bound to recombinant human FKBP. Ascomycin, also known as FR-

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